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Selective Plating Medium for Quantitative Recovery of Food-Borne *Listeria monocytogenes*

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A new plating medium (lithium chloride-ceftazidime agar [LCA]) was designed to quantitatively recover food-borne *Listeria monocytogenes* in the form of large colonies while inhibiting most other food-borne microorganisms. This medium included brain heart infusion agar as the nutritive agar base and a combination of selective agents (LiCl, glycine anhydride, and ceftazidime). Comparison of LCA and lithium chloride-phenylethanol-moxalactam agar (LPM) indicated that both were equally effective for the enumeration of the cold-tolerant pathogen in artificially and naturally contaminated foods. However, LCA was more effective than LPM in the recovery of sublethally heat-injured cells. Moreover, *Listeria* colonies on LCA exhibited a more distinct bluish hue than those on LPM when viewed by the Henry oblique transillumination technique.

The potential for outbreaks of listeriosis from foods contaminated with *Listeria monocytogenes* has been recognized recently. This has raised concern regarding the adequacy of methods for the quantitative detection of the cold-tolerant pathogen in foods (21). Consequently, considerable effort has been expended recently in the development and evaluation of selective agar media (2–5, 8, 11, 12, 17, 20). A major concern has been the complications associated with enumerating *L. monocytogenes* in foods containing high levels of other microorganisms. Typically, cells of this pathogen emerge as small colonies (<2.0 mm in diameter) on most plating media after 40 h at 30 to 35°C. This is certainly true with lithium chloride-phenylethanol-moxalactam agar (LPM [11]), which seems to be the most commonly used plating medium for detection of *L. monocytogenes* in various foods (3, 4, 6, 12).

Subsequent recognition and identification of suspected *L. monocytogenes* colonies take 3 to 4 weeks to complete (13). Datta et al. (6) succeeded in reducing the length of this step to 2 days by exploiting a colony hybridization technique. One disadvantage of this approach is the use of a rapidly decaying radioisotope.

A preliminary study (R. V. Lachica, Abstr. Comprehensive Conf. *Listeria monocytogenes*, abstr. no. P5, 1988) described the formulation of a plating medium, lithium chloride-ceftazidime agar (LCA), for the quantitative detection of *L. monocytogenes* in foods. The medium was designed to facilitate the formation of large colonies of the pathogen in order to permit rapid identification without the need for special equipment or unstable reagents. In the present study, LCA was compared with LPM by using artificially and naturally contaminated retail-level foods as well as sublethally heat-stressed broth cultures as test materials.

MATERIALS AND METHODS

Cultures. *L. monocytogenes* Scott A, V7, and CA were maintained on slants of heart infusion agar (Difco Laboratories) at 4°C; fresh cultures were grown in brain heart infusion (BHI) broth (Difco) at 35°C for 24 h. To obtain heat-injured cells, these broth cultures were preheated at 50°C for 60 min and cooled rapidly to room temperature.

Food samples. All food samples were purchased at a local supermarket, transported to the laboratory, stored at 4°C, and analyzed within 24 h. Some samples which were negative for *L. monocytogenes*, i.e., <100 CFU/g, were stored for 2 months at 4°C before being artificially contaminated with *Listeria* cultures at ca. 10^3 CFU/g.

Media. LPM was prepared as described by Lee and McClain (11). LCA was prepared by adding the following to a liter of BHI agar (BHIA): 5 g of LiCl, 10 g of glycine anhydride, and 2.5 ml of filter-sterilized solution containing 50 mg of ceftazidime pentahydrate (Glaxo Pharmaceuticals, Ltd.). Plate count agar (Difco) was used to obtain the aerobic plate count (30°C for 48 h) of the stored food samples.

Direct plating. A 10^{-1} dilution was prepared by mixing 10 g of each food sample with 90 ml of sterile 0.1% peptone water in a stomacher bag. The food suspension was blended in a stomacher device (Dynatech Laboratories, Inc.) for 2 min. Two 0.1-ml portions of the diluted samples were inoculated onto duplicate prepoured plates of LCA, LPM, and plate count agar by using sterile bent glass rods. In a similar manner, the heat-stressed cultures were decimally diluted and plated in duplicate onto LCA, LPM, and BHIA.

Recognition of *Listeria* colonies. After incubation for 40 h at 30°C, the plates were scanned for typical *Listeria* colonies by the Henry oblique transmitted light viewing technique (9, 10) as well as by using reflected light. A ruler was used to measure the diameter of the *Listeria* colonies.

Identification. Definitive identification of *L. monocytogenes* colonies was performed by the scheme suggested by McClain and Lee (14).

RESULTS

Artificially contaminated foods. LCA and LPM were compared for their ability to recover *L. monocytogenes* from the contaminating background flora of selected foods. Both media gave comparable results in facilitating high recovery of *Listeria* colonies (Table 1). Despite the presence in the samples of large numbers of microflora (aerobic plate count, $>10^8$ CFU/g), there was no difficulty in recognizing the colonies that emerged from *Listeria* cells inoculated at ca. 10^3 CFU/g. Most of the other bacteria that formed as background colonies on LCA were streptococci and staphylococci. The iridescence of these colonies was distinctly different from the bluish hue of the *Listeria* colonies when colonies were viewed by oblique transmitted light. Cells of *L. monocytogenes* emerged as large colonies on LCA (ca.

TABLE 1. Ability of LCA and LPM to quantitatively recover moderate levels of *L. monocytogenes* from heavily contaminated foods[a]

Food	% Recovery[b] on:	
	LCA	LPM
Ham	121 ± 50	98 ± 63
Canadian bacon	106 ± 14	100 ± 10
Turkey ham	141 ± 38	164 ± 33
Smoked chicken breast	91 ± 7	110 ± 12
Turkey salami	82 ± 26	76 ± 23
Pork sausage	90 ± 28	115 ± 7

[a] Foods with a microbial load of $>1 \times 10^8$ CFU/g were each inoculated with *L. monocytogenes* at ca. 10^3 CFU/g.
[b] Values (means ± standard deviation) are composites of the recovery of strains Scott A, V7, and CA for each type of food.

2.5 mm in diameter), whereas colonies on LPM were small (ca. 1.5 mm in diameter).

Naturally contaminated foods. In the course of assessing the ability of LCA to inhibit the background food-borne microflora, one food sample turned out unexpectedly to be positive for *L. monocytogenes* (>100 CFU/g). Consequently, the decision was made to screen several food samples for *Listeria* contamination by direct plating of LCA and LPM. Contamination of retail-level foods by *L. monocytogenes* at levels detectable by direct plating appeared to be highly sporadic. Five naturally contaminated food samples were used to compare the efficacy of LCA with LPM for the enumeration of *L. monocytogenes* (Table 2). Both media were essentially comparable. LPM had fewer and smaller background colonies as well as smaller *Listeria* colonies. On the other hand, LCA promoted the formation of larger *Listeria* colonies (ca. 2.5 mm in diameter) whose bluish hue was more distinct than those on LPM when colonies were viewed by the Henry oblique transillumination technique.

Heat-stressed cultures. The efficiency of LCA and LPM in recovering sublethally heat-stressed cells of *L. monocytogenes* was compared with that of BHIA, a rich medium noted for its ability to support growth of many fastidious microorganisms. LCA and BHIA gave comparable results, whereas only 15% or fewer colonies were recovered on LPM (Table 3). The colonies on LCA and BHIA varied in size from 1.0 to 2.5 mm in diameter and were generally larger than those on LPM.

DISCUSSION

The results presented here indicate the ability of LCA to promote the formation of large colonies of *L. monocytogenes* and at the same time suppress the growth of most of the other food-borne microflora. The medium provides a nutritious environment; its nutritive agar base (BHIA) contains glucose, which is considered the best energy source for the growth of *Listeria* species (16, 19). Most published formulations of plating media do not contain any added carbohydrates that can be utilized by *L. monocytogenes*. Some media do contain esculin as a diagnostic test (17, 20). However, it is not used as efficiently as glucose by *L. monocytogenes*, as pointed out by Swaminathan et al. (19). Among the various glucose-containing basal media examined, none could match the ability of BHIA to promote good growth of *L. monocytogenes* (data not shown).

TABLE 2. Quantitative recovery of *L. monocytogenes* from naturally contaminated retail food samples

Food	Detection level (CFU/g) on:	
	LCA	LPM
Oyster	1.4×10^3	5.5×10^2
Brie cheese	6.0×10^2	2.5×10^2
Chicken roll	1.8×10^5	1.9×10^5
Pork sausage	6.0×10^2	4.0×10^2
Sliced beef, precooked	1.0×10^2	$<10^2$

TABLE 3. Efficiency of LCA and LPM in recovering heat-stressed *Listeria monocytogenes* cells

Strain	Recovery (10^7 CFU/ml) (%) on[a]:	
	LCA	LPM
Scott A	22.5 (78.6)	2.5 (8.9)
V7	96.0 (104)	14.3 (15.2)
CA	49.5 (102)	2.5 (5.0)

[a] Each value represents the mean of two replicates in duplicate. Percent recoveries were calculated by using CFU per milliliter from BHIA as 100%.

The choice of selective agents for LCA was aided immensely by an extensive survey by Smith and Archer (18) of the degrees to which selective agents inhibit the recovery of sublethally heat-injured *Listeria* cells. The assumption was made in the current study that agents highly inhibitory to injured cells are also likely to have a negative effect on the growth of healthy cells, resulting in the formation of small colonies. Consequently, the choice of selective agents was sharply focused on a few compounds. In combination with LiCl and glycine anhydride, ceftazidime and moxalactam were generally comparable in their effectiveness in controlling the background microflora. However, ceftazidime was slightly more permissive to *L. monocytogenes* and more inhibitory to the other food-borne bacteria. The use of tellurite reduction as a diagnostic test was abandoned when potassium tellurite levels as low as 0.001% were found to be inhibitory to heat-injured *Listeria* cells. Moreover, the occasional staphylococci, streptococci, and bacilli that emerged on LCA were also capable of reducing tellurite.

The use of naturally contaminated food samples as test materials provided convincing evidence of the efficacy of LCA for the quantitative detection of food-borne *L. monocytogenes*. Moreover, the results from the artifically contaminated samples further indicated that the presence of an overwhelming level of background microflora is not a problem with either LCA or LPM. The problem is with food samples with levels of listerial contamination below the sensitivity of the direct plating technique (<100 CFU/g); these samples require a recovery and a selective-enrichment phase prior to plating.

The unique ability of LCA to efficiently recover healthy and sublethally injured *L. monocytogenes* cells is an advantage that can be useful for the recovery and enumeration of stressed cells, a portion of which may consist of sublethally injured cells in certain foods, such as pasteurized milk (7). LPM and other selective media are too inhibitory to stressed cells of *L. monocytogenes* (18). In addition, LCA appears to support growth of all *Listeria* species, whereas *L. seeligeri* and *L. welshimeri* grow on LPM with difficulty (data not shown). These two species have been reported recently to cause infection in humans (1, 15). Of greater significance is the ability of LCA to promote the formation of large *Listeria* colonies and still control the growth of the background microflora. This is critical for the next phase of the study,

which is the development of a same-day identification of *L. monocytogenes* colonies (R. V. Lachica, manuscript in preparation).

The oblique transmitted light viewing technique proved invaluable for the initial recognition of *Listeria* colonies. It obviated the need to incorporate a diagnostic test that could complicate the chemical makeup of LCA. Nevertheless, investigators have had some difficulties with the technique (4, 17, 20), which is quite cumbersome. Consequently, a modified version of the technique that is more reproducible and convenient than the current version is being developed in this laboratory.

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